



Image-processing methods for electron microscopy of biological specimens

Carlos Oscar S. Sorzano,^{a*} Alberto Bartesaghi^{b*} and Amit Singer^{c*}

^aCentro Nacional de Biotecnología–CSIC, C/Darwin 3, Cantoblanco, 28049 Madrid, Spain, ^bDepartment of Biochemistry, Duke University School of Medicine, Durham, NC 27708, USA, and ^cDepartment of Mathematics and Program in Applied and Computational Mathematics, Princeton University, Princeton, NJ 08540, USA. *Correspondence e-mail: coss@cnb.csic.es, alberto@cs.duke.edu, amits@math.princeton.edu

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Cryo-electron microscopy (cryoEM) has become a cornerstone in structural biology, enabling the determination of macromolecular structures at increasingly high resolutions and in native environments. The success of cryoEM stems from advances across multiple domains, including electron optics, detector technology and sample preparation, and the continuous evolution of image-processing methods. Algorithmic innovation is critical in pushing the boundaries of what is structurally and functionally observable at the molecular scale.

In recognition of the rapid methodological developments in the field, a series of special issues has been dedicated to image-processing methods for electron microscopy of biological specimens. The first two editions were published in *Biological Imaging* (Cambridge University Press) in 2023 and 2024, as part of a broader effort to highlight algorithmic advances driving progress in structural biology (<https://www.cambridge.org/core/journals/biological-imaging/special-collections/image-processing-applications-for-electron-microscopy-of-biological-specimens>). Building on the success and community interest generated by these volumes, the initiative has now been adopted by *Acta Crystallographica Section D*, a leading journal in structural biology, where the current edition can be found (https://journals.iucr.org/special_issues/2025/imageprocessing).

A key goal of this initiative is to establish a stable and recurring venue for disseminating innovations in cryoEM image processing. Rather than organizing isolated, sporadic special issues, this annual series provides the community with a reliable and well-timed opportunity to present new methods, share open-source tools and report benchmarking results. The continuity of hosting the special issue in the same journal each year reinforces its visibility, enhances its impact and encourages sustained engagement from authors, reviewers and readers alike.

This special issue welcomes contributions describing novel algorithms, software tools, reference data sets and methodological advances across the spectrum of cryoEM. Topics of interest include, but are not limited to, single-particle analysis, electron tomography, electron crystallography and microcrystal electron diffraction. Submissions addressing conformational heterogeneity, integrative modeling or connections with complementary imaging techniques are also encouraged.

The call for papers follows a two-stage submission process. In the first stage, authors are asked to submit a short white paper (a maximum of two pages, free format) by 1 July every year. Authors of selected white papers are then invited to submit a full manuscript through the journal's editorial system. The peer review and publication process are scheduled for completion early the following year.

This year's special issue features the following articles.

TomoCPT: a generalizable model for 3D particle detection and localization in cryo-electron tomograms (Shah *et al.*, 2025) introduces a transformer-based tool designed to enhance particle detection in cryo-electron tomography (cryo-ET). By reformulating particle detection as a centroid-prediction task using Gaussian labels, *TomoCPT* offers improved accuracy over traditional binary labeling and template-matching methods. Built upon the SwinUNETR architecture, the model demonstrates strong generalization capabilities, including zero-shot inference, and benefits from fine-tuning with limited data.

The *ManifoldEM* method for cryo-EM: a step-by-step breakdown accompanied by a modern Python implementation (Ojha *et al.*, 2025) presents a detailed and accessible guide to a computational technique designed to analyze continuous conformational heterogeneity in single-particle cryo-EM data. Unlike traditional approaches that model macromolecular dynamics as discrete states, *ManifoldEM* captures smooth structural transitions, offering richer insights into molecular function. The authors provide a modern Python-based implementation of the method and demonstrate its utility on several experimental data sets, enabling the visualization of conformational landscapes and dynamic pathways directly from experimental cryo-EM images.

InstaMap: instant-NGP for cryo-EM density maps (Woollard *et al.*, 2025) presents a novel method that adapts the instant-NGP framework from computer vision to represent cryo-EM density maps directly in real space using a multi-resolution hash encoding. Unlike traditional Fourier-based

approaches, *InstaMap* enables more intuitive manipulation of density maps and simplifies tasks such as masking and local resolution estimation. The method demonstrates high-quality reconstructions on synthetic and experimental data sets and shows potential for resolving conformational heterogeneity. By bridging neural graphics primitives and cryo-EM, *InstaMap* offers a promising direction for more flexible and efficient 3D reconstructions in structural biology.

References

- Ojha, A. A., Blackwell, R., Cruz-Chú, E. R., Dsouza, R., Astore, M. A., Schwander, P. & Hanson, S. M. (2025). *Acta Cryst.* **D81**, 89–104.
- Shah, P. N. M., Sanchez-Garcia, R. & Stuart, D. I. (2025). *Acta Cryst.* **D81**, 63–76.
- Woollard, G., Zhou, W., Thiede, E. H., Lin, C., Grigorieff, N., Cossio, P., Dao Duc, K. & Hanson, S. M. (2025). *Acta Cryst.* **D81**, 147–169.